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Contractor

LITTON BIONETICS INC

Document Title

MUTAGENICITY EVALUATION OF FHP 3002 LIQUID PREPOLYMER IN THE AMES SALMONELLA/MICROSOME PLATE TEST (FINAL REPORT) WITH COVER LETTER DATED 011691

Chemical Category

TOLUENE DIISOCYANATE (26471-62-5)

CONTAINS NO CBI

GRACE 86-910000647

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91 JAN 24 AM 9:44

January 16, 1991

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Attn: Health and Safety Reporting Rule (Notification/Reporting)

Please find attached 8(d) health and safety reports for mixtures processed containing toluene diisocyanate (CAS #26471-62-5), 4,4-Diphenylmethane diisocyanate (CAS #101-68-8) and 1,6-Diisocyanatohexane (CAS #822-06-0). Grace is submitting these reports for late filing since their submittal may have been subject to the isocyanates 10-year call-in of June 1, 1987.

We have reason to believe that some of these reports may have previously been submitted to EPA as attachments to PMN submissions. However, Grace is filing them as a precautionary measure to insure EPA's receipt.

These reports are being submitted for:

W. R. Grace & Co.-Conn.
Washington Research Center
7379 Route 32
Columbia, MD 21044

Sincerely,

J. W. Raksis

A:\JR91-013/lw

Attachments - 20

86910000638

CONTAINS NO CBI

GENETICS ASSAY NO. 5124

LBI SAFETY NO. 6008

Toluene Diisocyanate
26471-62-5
1,6-Diisocyanatohexane
822-06-0

MUTAGENICITY EVALUATION OF

FHP 3002 LIQUID PREPOLYMER

IN THE
AMES SALMONELLA/MICROSOME
PLATE TEST

FINAL REPORT

SUBMITTED TO:

W.R. GRACE AND CO.
7379 ROUTE 32
COLUMBIA, MD 21044

8691000064?

86910000647

SUBMITTED BY:

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 20988

REPORT DATE: JULY, 1980

Litton BIONETICS

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PREFACE

This report contains a summary of the data compiled during the evaluation of the test compound. The report is organized to present the results in a concise and easily interpretable manner. The first part contains items I-IX. Items I-IV provide sponsor and compound identification information, type of assay, and the protocol reference number. All protocol references indicate a standard procedure described in the Litton Bionetics, Inc. "Screening Program for the Identification of Potential Mutagens and Carcinogens." Item V provides the initiation and completion dates for the study, and Item VI provides identification of supervisory personnel. Item VII identifies the tables and/or figures containing the data used by the study director in interpreting the test results. The interpretation itself is in Item VIII. Item IX provides the conclusion and evaluation.

The second part of the report describes the materials and procedures employed in conducting the assay. This part of the report also contains evaluation criteria used by the study director, and any appendices. The evaluation criteria are included to acquaint the sponsor with the methods used to develop and analyze the test results.

All test and control results presented in this report are supported by fully documented raw data which are permanently maintained in the files of the Department of Genetics and Cell Biology or in the archives of Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, Maryland 20795.

I. SPONSOR: W.R. Grace and Co.

II. MATERIAL (TEST COMPOUND): GENETICS ASSAY NUMBER: 5124

A. Identification: FHP 3002 liquid Prepolymer

B. Date Received: May 15, 1980

C. Physical Description: Viscous yellow liquid

III. TYPE OF ASSAY: Ames Salmonella/microsome Mutagenesis Assay

IV. PROTOCOL NUMBER: 401

V. STUDY DATES:

A. Initiation: May 30, 1980

B. Completion: June 17, 1980

VI. SUPERVISORY PERSONNEL:

A. Study Director: D.R. Jagannath, Ph.D.

B. Laboratory Supervisor: Sibyl Goode

VII. RESULTS:

The results of this assay are presented in Table 1.

VIII. INTERPRETATION OF RESULTS:

The test compound was examined for mutagenic activity in a series of in vitro microbial assays employing Salmonella indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats.

DOSE RANGE

The dose range employed for the evaluation of this compound was from 0.5 µg to 5000 µg per plate.

TOXICITY

The test material did not exhibit toxicity with any of the indicator strains used in this assay.

VIII. INTERPRETATION OF RESULTS (continued):

The results of the tests conducted on the test material in the absence of a metabolic activation system were negative. The tests with TA-1538, TA-98 and TA-100 were repeated because of the low number of revertants observed at various test doses in the initial assay. The repeat tests were negative.

The results of the tests conducted on the test material in the presence of a rat liver activation system were negative. The test with TA-1535 was repeated because of the increase in the number of revertants observed at 0.5 µg dose level in the initial assay. The tests with TA-1538 and TA-100 were repeated because of the low number of revertants observed at various test doses in the initial assay. The repeat tests were negative.

IX. CONCLUSIONS:

The test material FHP 3002 liquid Prepolymer did not exhibit genetic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions according to our evalution criteria.

Submitted by:

Study Director

D.R. Jagannath 7/22/80

D.R. Jagannath, Ph.D. Date
Section Chief
Submammalian Genetics
Department of Genetics
and Cell Biology

Reviewed by:

David J. Brusick 7/22/80

David J. Brusick, Ph.D. Date
Director
Department of Genetics
and Cell Biology

Litton BIONETICS

RESULTS

TABLE 1

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: FHP 3002 PSF POLYMER LIQUID
B. SOLVENT: DIST H2O
C. TEST INITIATION DATES: 05/30/80 06/05/80
D. TEST COMPLETION DATE: 06/17/80
E. S-9 LOT#: 08009
NOTE: CONCENTRATIONS ARE GIVEN IN MICROGRAMS

TEST	SPECIES	TISSUE	REVERTANTS PER PLATE													
			TA-1535			TA-1537			TA-1538			TA-98			TA-100		
			1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
NONACTIVATION																	
SOLVENT CONTROL	---	---	22			7			12	14		18	27		149	88	
SOLVENT CONTROL	---	---	24			10			12	17		30	27		181	98	
POSITIVE CONTROL**	---	---	1021			206			816	1573		815	998		1053	942	
POSITIVE CONTROL**	---	---	1026			161			1349	1657		970	741		1094	876	
TEST COMPOUND																	
0.500000 UG	---	---	32			8			7	17		4	22		22	104	
1.000000 UG	---	---	16			8			12	18		8	30		69	99	
10.000000 UG	---	---	16			12			7	14		23	29		85	93	
100.000000 UG	---	---	18			6			6	18		21	28		84	105	
500.000000 UG	---	---	16			11			4	12		26	20		94	102	
1000.000000 UG	---	---	28			8			6	18		18	26		113	101	
2500.000000 UG	---	---	-			-			-	-		16	21		134	96	
5000.000000 UG	---	---	-			-			-	-		20	19		21	106	
ACTIVATION																	
SOLVENT CONTROL	RAT	LIVER	33	15		12			26	24		31			109	72	
SOLVENT CONTROL	RAT	LIVER	38	12		14			28	25		40			169	105	
POSITIVE CONTROL***	RAT	LIVER	285	159		200			1191	1977		1680			1555	1491	
POSITIVE CONTROL***	RAT	LIVER	293	142		231			1249	1889		1762			1688	1391	
TEST COMPOUND																	
0.500000 UG	RAT	LIVER	41	14		12			1	23		32			1	101	
1.000000 UG	RAT	LIVER	26	12		13			0	22		41			21	102	
10.000000 UG	RAT	LIVER	21	15		8			0	24		33			34	113	
100.000000 UG	RAT	LIVER	15	11		11			3	26		53			41	108	
500.000000 UG	RAT	LIVER	16	12		12			5	26		55			60	111	
1000.000000 UG	RAT	LIVER	38	18		9			0	17		40			66	92	
2500.000000 UG	RAT	LIVER	-	-		-			-	-		41			60	95	
5000.000000 UG	RAT	LIVER	-	-		-			-	-		46			1	108	

**

TA-1535	SODIUM AZIDE	10 UG/PLATE
TA-1537	9-AMINOACRIDINE	50 UG/PLATE
TA-1538	2-NITROFLUORENE	10 UG/PLATE
TA-98	2-NITROFLUORENE	10 UG/PLATE
TA-100	SODIUM AZIDE	10 UG/PLATE
SOLVENT	50 UL/PLATE	

***

TA-1535	2-ANTHRAMINE	2.5 UG/PLATE
TA-1537	2-ANTHRAMINE	2.5 UG/PLATE
TA-1538	2-ANTHRAMINE	2.5 UG/PLATE
TA-98	2-ANTHRAMINE	2.5 UG/PLATE
TA-100	2-ANTHRAMINE	2.5 UG/PLATE

- INDICATES TEST WAS NOT DONE

AMES SALMONELLA/MICROSOME PLATE ASSAY

1. OBJECTIVE

The objective of this study is to evaluate a test material for mutagenic activity in a bacterial assay with and without a mammalian S9 activation system.

2. RATIONALE

The Salmonella typhimurium strains used at LBI are all histidine auxotrophs by virtue of mutations in the histidine operon. When these histidine-dependent cells are grown in a minimal media petri plate containing a trace of histidine, only those cells that revert to histidine independence (his+) are able to form colonies. The trace amount of histidine allows all the plated bacteria to undergo a few divisions; this growth is essential for mutagenesis to occur. The his+ revertants are easily scored as colonies against the slight background growth. The spontaneous mutation frequency of each strain is relatively constant; but when a mutagen is added to the agar, the mutation frequency is increased 2- to 100-fold. Cells which grow to form colonies on the minimal media petri plates are therefore assumed to have reverted, either spontaneously or by the action of a test substance to his+ genotype.

3. MATERIALS

A. Indicator Microorganisms

The Salmonella typhimurium strains used in this assay were obtained from Dr. Bruce Ames, University of California at Berkeley.[1-5] The following 5 strains are routinely used.

Strain Designation	Gene Affected	Additional Mutations			Mutation Type Detected
		Repair	LPS	R Factor	
TA-1535	his G	Δ uvr B	rfa	-	Base-pair substitution
TA-1537	his C	Δ uvr B	rfa	-	Frameshift
TA-1538	his D	Δ uvr B	rfa	-	Frameshift
TA-98	his D	Δ uvr B	rfa	pKM101	Frameshift
TA-100	his G	Δ uvr B	rfa	pKM101	Base-pair substitution

All of the above strains have, in addition to the mutation in the histidine operon, a mutation (rfa-) that leads to defective lipopolysaccharide coat, a deletion that covers genes involved in the synthesis of vitamin biotin (bio-) and in the repair of ultraviolet (uv) - induced DNA damage (uvrB-). The rfa- mutation makes the strains more permeable to many large molecules. The uvrB- mutation decreases repair of some types of chemically or physically damaged DNA and thereby enhances the strain's sensitivity to some mutagenic agents. The resistant transfer factor plasmid (R factor) pKM101, in TA-98 and TA-100 is believed to cause an increase in error-prone DNA repair that leads to many more mutations for a given dose of most mutagens[5]. In addition, plasmid pKM101 confers resistance to the antibiotic ampicillin, which is a convenient marker to detect the presence of plasmid in the cells.

All indicator strains are kept at 4°C on minimal medium plates supplemented with a trace of biotin and an excess of histidine. The plates with plasmid-carrying strains contain in addition ampicillin (25 µg/ml), to ensure stable maintenance of plasmid pKM101. New stock culture plates are made every two months from the frozen master cultures or from single colony reisolates that were checked for their genotypic characteristics (his, rfa, uvrB, bio) and for the presence of plasmid. For each experiment, an inoculum from the stock culture plates is grown overnight at 37°C in nutrient broth (Oxoid CM67) and used.

B. Media

The bacterial strains were cultured in Oxoid Media #2 (nutrient Broth). The selective medium was Vogel Bonner Medium E with 2% glucose[7]. The overlay agar will consist of 0.6% purified agar with 0.5 mM histidine, 0.05 mM biotin and 0.1M NaCl according to the methods of Ames et al.[6]

C. Activation System

(1) S9 Homogenate

A 9,000 x g supernatant prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 (described by Ames et al.[6]) was purchased from Bionetics Laboratory Products, Litton Bionetics, Inc. and used in this assay.

(2) S9 Mix

Components	Concentration per Milliliter S9 Mix
NADP (sodium salt)	4 µmoles
D-glucose-6-phosphate	5 µmoles
$MgCl_2$	8 µmoles
KCl	33 µmoles
Sodium phosphate buffer pH 7.4	100 µmoles
Organ homogenate from rat liver (S9 fraction)	100 µliters

4. EXPERIMENTAL DESIGN

A. Dosage Selection

All tests are run at a minimum of four concentrations. In the standard plate test, at least six dose levels of the test material, dissolved in a suitable solvent, are added to the test system. The standard test doses are 0.005, 0.01, 0.1, 1.0, 5.0 and 10.0 µliters per plate for liquids and 0.5, 1.0, 10.0, 100.0, 500.0 and 1000.0 µg per plate for solids. Additional doses may be employed in the tests if toxicity is observed at the three highest doses. When no toxicity is observed, additional concentrations may be employed up to 50 µliters or 5000 µg per plate.

B. Mutagenicity Testing

The procedure used is based on the paper published by Ames et al.[6] and is performed as follows:

(1) Nonactivation Assay

To a Sterile 13 x 100 mm test tube placed in a 43°C water bath the following is added in order:

(a) 2.00 ml of 0.6% agar containing 0.05 mM histidine and 0.05 mM biotin.

(b) 0.05 ml of a solution of the test chemical to give the appropiate dose.

(c) 0.1 ml - 0.2 ml of indicator organism/s.

(d) 0.50 ml of 0.01M phosphate buffer, pH 7.4.

This mixture is swirled gently and then poured nto minimal agar plates (see 3B, Media). After the top agar has set, the plates are incubated at 37°C for approximately 2 days. The number of his+ revertant colonies growing on the plates is counted and recorded.

(2) Activation Assay

The activation assay is run concurrently with the nonactivation assay. The only difference is the addition of 0.5 ml of S9 mix (see 3C:2, Activation System) to the tubes in place of 0.5 ml of phosphate buffer which is added in nonactivation assays. All other details are similar to the procedure for nonactivation assays.

A detailed flow diagram for the plate incorporation assay is provided in Figure 1.

C. Control Compounds

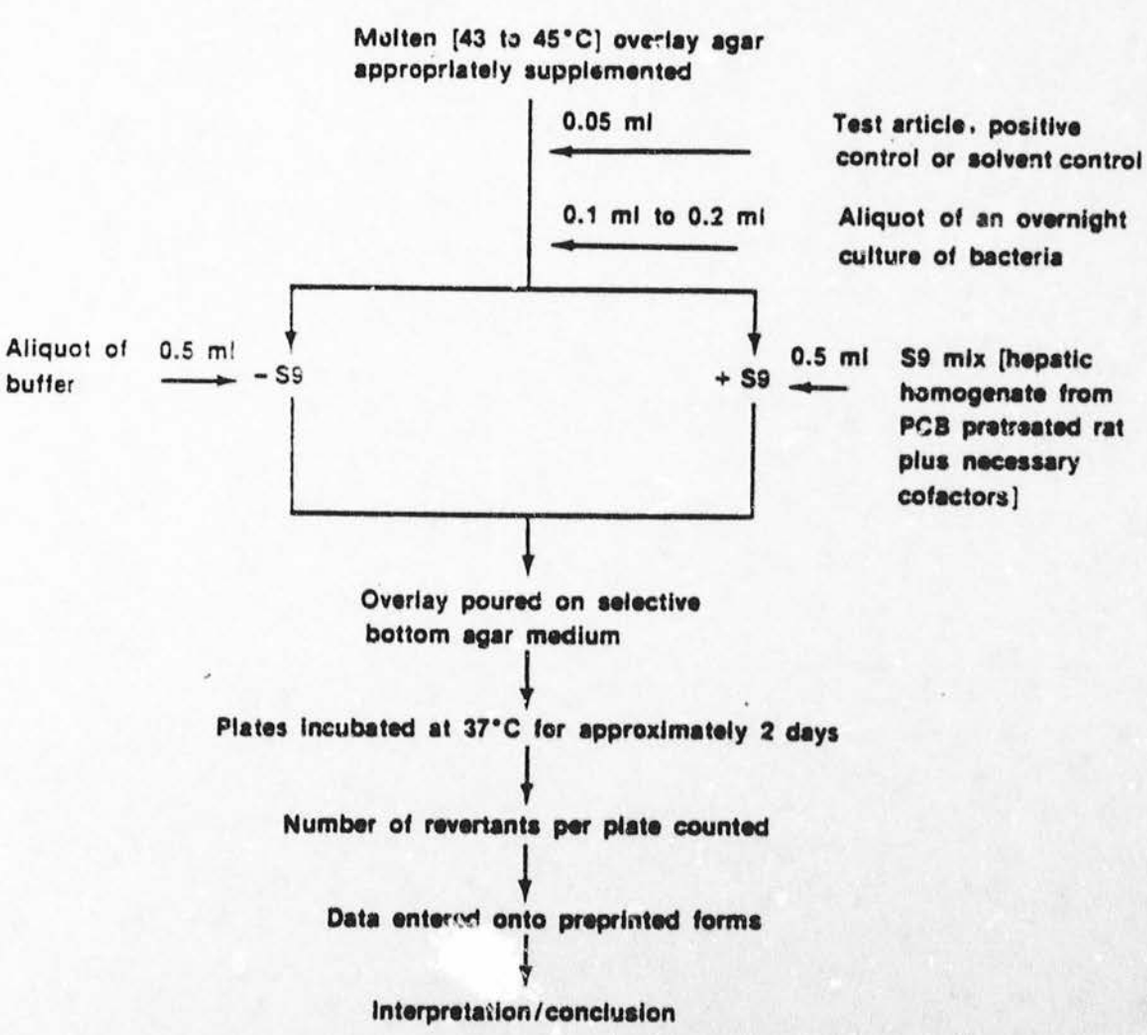
A negative control consisting of the solvent used for the test material is performed in all cases. For negative controls, step '5' of Nonactivation Assays is replaced by 0.05 ml of the solvent. The negative controls are employed for each indicator strain and is performed in the absence and presence of S9 mix. The solvent used to prepare the stock solution of the test material is given in the Results section of this report. All dilutions of the test material made using this solvent.

Specific positive control compounds known to revert each strain are also used in the assays. The concentrations and specificities of these compounds to specific strains are given in the following table.

Assay	Chemical	Solvent	Concentration per Plate (µg)	Salmonella Strains
Nonactivation	Sodium azide	Water	10	TA-1535, TA-100
	2-Nitrofluorene (NF)	Dimethyl-sulfoxide	10	TA-1538, TA-98
	9-aminoacridine (9AA)	Ethanol	50	TA-1537
Activation	2-anthramine (ANTH)	Dimethyl-sulfoxide	2.5	For all strains

FIGURE 1

REVERSE MUTATION ASSAY
(Agar Incorporation Method)



5. EVALUATION CRITERIA

Statistical methods are not currently used, and evaluation is based on the criteria included in this protocol.

Plate test data consists of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test material and the cells are incubated in the overlay for approximately 2 days and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test:

- The small number of cell divisions permits potential mutagens to act on replication DNA, which is often more sensitive than nonreplicating DNA.

- The combined incubation of the test article and the cells in the overlay permits constant exposure of the indicator cells for approximately 2 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test material, the surviving population on the treatment plates is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol will normally employ several doses ranging over two or three log concentrations. This does not apply to spot tests and tests performed on fabrics and like materials which are tested at a single concentration.

B. Dose-Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. A factor that might modify dose-response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test material may kill any mutants that are induced, and the test material will not appear to be mutagenic.

C. Control Tests

Positive and negative control assays will be conducted with each experiment and will consist of direct-acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls will consist of the test material solvent in the overlay agar together with the other essential components. The negative control plate for each strain will give a reference point to which the test data will be compared. The positive control assay will be conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures to be used to evaluate the mutagenicity of the test material are semiquantitative, the criteria to be used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets will be evaluated using the following criteria.

(1) Strains TA-1535, TA-1537 and TA-1538

If the solvent control value is within the normal range, a test material that produces a positive dose response over three concentrations with the highest increase equal to three times the solvent control value will be considered to be mutagenic.

(2) Strains TA-98 and TA-100

If the solvent control value is within the normal range, a test material that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-98 and TA-100 will be considered to be mutagenic.

(3) Pattern

Because TA-1535 and TA-100 are both derived from the same parental strain (G-46) and because TA-1538 and TA-98 are both derived from the same parental strain (D3052), to some extent there is a built-in redundancy in the microbial assay. In general, the two strains of a set respond to the same mutagen and such a pattern is sought. Generally, if a strain responds to a mutagen in nonactivation tests, it will do so in activation tests.

(4) Reproducibility

If a test material produces a response in a single test that cannot be reproduced in additional runs, the initial positive test data lose significance.

E. Evaluation Criteria for Toxicity

(1) Complete toxicity

When there are no revertants observed on the plate(s) treated with the test compound, the test compound will be defined as toxic to all or any of the indicator strains at that particular dose(s).

(2) Slight toxicity

When there are fifty per cent or less number of revertants on the plate(s) treated with the test compound as compared to the solvent control plate(s), the test compound will be defined as slightly toxic to all or any of the indicator strains at that particular dose(s).

F. Relation Between Mutagenicity and Carcinogenicity

It must be emphasized that the Ames Salmonella/Microsome Plat Assay is not a definitive test for chemical carcinogens. It is recognized, however, that correlative and functional relations have been demonstrated between these two endpoints. The results of comparative tests on 300 chemicals by McCann et al.[2] show an extremely good correlation between results of microbial mutagenesis tests and in vivo rodent carcinogenesis assays.

All evaluations and interpretation of the data to be presented in the final report will be based only on the demonstration, or lack, of mutagenic activity.

REFERENCES

1. J. McCann, E. Choi, E. Yamasaki, and B.N. Ames. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc. Nat. Acad. Sci. USA 72, 5135-5139 (1975).

2. B.N. Ames, E.G. Gurney, J.A. Miller, and H. Bartsch. Carcinogens as frameshift mutagens: Metabolites and derivatives of 2-acetylaminofluorene and other aromatic amine carcinogens. Proc. Nat. Acad. Sci. USA 69, 3128-3132 (1972).

3. B.N. Ames, F.D. Lee, and W.E. Durston. An improved bacterial test system for the detection and classification of mutagens and carcinogens. Proc. Nat. Acad. Sci. USA 70, 782-786 (1973).

4. B.N. Ames, W.E. Durston, E. Yamasaki, and F.D. Lee. Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. Proc. Nat. Acad. Sci. USA 70, 2281-2285 (1973).

5. J. McCann, N.E. Springarn, J. Kobori, and B.N. Ames. Detection of carcinogens as mutagens: Bacterial tester strains with R factor plasmids. Proc. Nat. Acad. Sci. USA 72, 979-983 (1975).

6. B.N. Ames, J. McCann, and E. Yamasaki. Methods for detecting carcinogens and mutagens with the Salmonella/mamallian-microsome mutagenicity test. Mutation Res. 31, 347-364 (1975).

7. H.J. Vogel and D.M. Bonner. Acetylornithinase of E. coli; Partial purification and some properties. J. Biol. Chem., 218, 97-106 (1956).

Q.A. Inspection Statement
(reference 21 CFR 58.35(b)(7))

PROJECT 20988 LBI Assay No. 5124

TYPE of STUDY Ames Plate Test

This final study report was reviewed by the LBI Quality Assurance Unit on July 24, 1980. A report of findings was submitted to the Study Director and to Management on July 29, 1980.

The short-term nature of this study precluded inspection while it was in process. The Quality Assurance Unit inspects an in-process study of this type approximately every three months to assure that no significant problems exist that are likely to affect the integrity of this type of study.

P M. Cuevas
Auditor, Quality Assurance Unit

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